Poaceae (or Gramineae) belong to the grass family and is one of the largest families among flowering plants on land. They include some of the most important cereal crops such as rice (*Oryza sativa*), barley (*Hordeum vulgare*), wheat (*Triticum aestivum*), maize (*Zea mays*), and sorghum (*Sorghum bicolor*). The characteristic bushy appearance of grass plants, including cereal crops, is formed by the activities of axillary meristems (AMs) generated in the leaf axil. These give rise to tillers from the basal nodes which recapitulate secondary growth axis and AMs are formed during vegetative development. On transition to flowering the apical meristem transforming to an inflorescence meristem (IM) which produces branches from axillary meristem. These IM gives rise to branches that ultimately bear florets. Vegetative branching/tillering determines plant biomass and influences the number of inflorescences per plant. While inflorescence branching determines the number of florets and hence seeds. Thus the overall activity of axillary meristems plays a key role in determining plant architecture during both vegetative and reproductive stages. In *Arabidopsis*, research on the plant specific transcription factor LEAFY (LFY) has pioneered our understanding of its regulatory functions during transition from vegetative to reproductive development and its role in specifying a floral meristem (FM) identity to the newly arising lateral meristems. In the FM LFY activates other FM genes and genes for floral organ patterning transcription factors. *LFY* is strongly expressed throughout the young floral meristems from the earliest stages of specification but is completely absent from the IM (Weigel et al., 1992). *LFY* expression can also be detected at low levels in the newly emerging leaf primordia during the vegetative phase, and these levels gradually increase until the floral transition (Blazquez et al., 1997; Hempel et al., 1997).

In rice, the LFY ortholog- *RFL/APO2* is expressed predominantly in very young branching panicles/ inflorescence meristems (Kyozuka et al., 1998; Prasad et al., 2003) while in the vegetative phase RFL is expressed at axils of leaves (Rao et al., 2008). In rice FMs expression is restricted to primordia of lodicules, stamens, carpels and ovules (Ikeda-Kawakatsu et al., 2012). Knockdown of RFL activity or

*loss of function* mutants show delayed flowering and poor panicle branching with reduced number of florets and lower fertility (Rao et al., 2008, Ikeda-Kawakatsu et al., 2012). In some genotypes reduced vegetative axillary branching is also compromised (Rao et al., 2008). On the other hand *RFL* overexpression leads to the early flowering, attributing a role as an activator for the transition of vegetative meristems to inflorescence meristems (Rao et al., 2008). Thus, RFL shows a distinct developmental expression profile, has unique mutant phenotypes as compared to *Arabidopsis* LFY thus indicating a divergence in functions. We have used various functional genomics approaches to investigate regulatory networks controlledby RFL in the vegetative axillary meristems and in branching panicles with florets. These regulatory effects influence tillering and panicle branching, thus contributing to rice plant architecture.

**RFL functions in axillary meristem**

Vegetative AMs are secondary shoot meristems whose outgrowth determines plant architecture. In rice, AMs form tillers from basal nodes and mutants with altered tillering reveal that an interplay between transcription factors and the phytohormones - auxin, strigolactone underpins this process. We probed the relationship between *RFL* and other factors that control AM development. Our findings indicate that the derangements in AM development that occur on *RFL* knockdown arise from its early effects during specification of these meristems and also later effects during their outgrowth of AM as a tiller. Overall, the derailments of both steps of AM development lead to reduced tillering in plants with reduced *RFL* activity. Our studies on the gene expression status for key transcription factor genes, genes for strigolactone pathway and for auxin transporters gave an insight on the interplay between *RFL*, *LAX1* and strigolactone signalling. Expression levels of *LAX1* and *CUC* genes, that encode transcription factors with AM specification functions, were modulated upon *RFL* knockdown and on induction of RFL:ΔGR fusion protein. Thus our findings imply a likely, direct activating role for RFL in

AM development that acts in part, through attaining appropriate *LAX1* expression levels. Our data place meristem specification transcription factors LAX1 and CUC downstream to RFL. *Arabidopsis* LFY has a predominant role in conferring floral meristem (FM) identity (Weigel et al., 1992; Wagner, 2009; Irish, 2010; Moyroud et al., 2010). Its functions in axillary meristems were not known until recently. The latter functions were uncovered with the new *LFYHARA* allele with only partial defects in floral meristem identity (Chahtane et al., 2013). This mutant allele showed LFY can promote growth of vegetative AMs through its direct target REGULATOR OF AXILLARY MERISTEMS1 (RAX1), a R2R3 myb domain factor (Chahtane et al., 2013). These functions for *Arabidopsis* LFY and RAX1 in AMs development are parallel to and redundant with the pathway regulated by LATERAL SUPPRESSOR (LAS) and REGULATOR OF AXILLARY MERISTEM FORMATION1 (ROX1) (Yang et al., 2012; Greb et al., 2003). Interestingly, ROX1 is orthologous to rice LAX1 and our data show *LAX1* expression levels in rice panicles and in culms with vegetative AMs is dependent on the expression status of *RFL*. Thus, we speculate that as compared to *Arabidopsis* AM development, in rice the LFY-dependent and LFY-independent regulatory pathways for AMs development are closely linked. In *Arabidopsis, CUC2* and *CUC3* genes in addition to their role in shoot meristem formation and organ separation play a role in AM development possibly by defining a boundary for the emerging AM. These functions for the *Arabidopsis CUC* genes are routed through their effects on *LAS* and also by mechanisms independent of *LAS* (Hibara et al., 2006; Raman et al., 2008). These data show modulation in RFL activity using the inducible RFL:ΔGR protein leads to corresponding expression changes in *CUC1/CUC2* and *CUC3* genes expression in culm tissues. Thus, during rice AM development the meristem functions of *RFL* and *CUC* genes are related.

Consequent to specification of AM the buds are kept dormant. Bud outgrowth is influenced by auxin and strigolactone signalling pathways. We investigated the transcript levels, in rice culms of genes involved in strigolactone biosynthesis and

perception and found the strigolactone biosynthesis gene *D10* and hormone perception gene are significantly upregulated in *RFL* knockdown plants. Further, bioassays were done for strigolactone levels, where we used arbuscular mycorrhiza colonization assay as an indicator for strigolactone levels in wild type plants and in *RFL* knockdown plants. These data validate higher strigolactone signalling in *RFL* knockdown plants. To probe the relationship between RFL and the strigolactone pathway we created plants knocked down for both *RFL* and *D3*. For comparison of the tillering phenotype of these double knockdown plants we created plants with *D3* knockdown alone. We observed reduced tillering in plants with knockdown of both *RFL* and *D3* as compared to the tiller number in plants with knockdown of *D3* alone. These data suggest that *RFL* acts upstream to *D3* of control bud outgrowth. As effects of strigolactones are influenced by auxin transport we studied expression of *OsPIN1* and *OsPIN3* in *RFL* knockdown plants. Their reduced expression was correlated with auxin deficiency phenotypes of the roots in *RFL* knockdown plants. These data in conjunction with observations on *OsPIN3* the gene expression modulation by the induction of RFL:ΔGR allow us to speculate on a relationship between RFL, auxin transport and strigolactones with regard to bud outgrowth. We propose that the low tillering phenotype of *RFL* knockdown plants arises from weakened PATS, consequent to low levels of PIN1 and PIN3, coupled with moderate increase in strigolactones. Taken together, our findings suggest functions for *RFL* during AM specification and tiller bud outgrowth.

**RFL functions in panicle branching**

Prior studies on phenotypes of *RFL* knockdown or loss of function mutants suggested roles for RFL in transition to flowering, inflorescence meristem development, emergence of lateral organs and floral organ development (Rao et al., 2008; Ikeda-Kawakatsu et al., 2012). It has been speculated that RFL acts to suppress the transition from inflorescence meristem to floral meristem through its interaction with APO1 (Ikeda-Kawakatsu et al., 2012). The downstream genes

regulated by RFL in these processes have not yet been elucidated. To identify direct targets of RFL in developing panicles we adopted ChIP-seq coupled with studies on gene expression modulation on induction of RFL. For the former we raised polyclonal anti-sera and chromatin from branching panicles with few florets. For gene expression modulation studies, we created transgenics with a T-DNA construct where an artificial miRNA against 3'UTR specifically knocked endogenous *RFL* and the same T-DNA had a second expression cassette for generation of a chemically inducible RFL-ΔGR protein that is not targeted by amiR RFL. Our preliminary ChIP-seq data in the wild type panicle tissues hints that RFL binds to hundreds of loci across the genome thus providing first glimpse of direct targets of RFL in these tissues. These data, while preliminary, were manually curated to identify likely targets that function in flowering, we summarize here some key findings. Our study indicates a role of RFL in flowering transition by activating genes like *OsSPL14* and *OsPRMT6a*. Recent studies indicate that OsSPL14 directly binds to the promoter of OsMADS56 or FTL1, the rice homologs of SOC1 and FT to promote flowering (Lu et al., 2013). As *RFL* knockdown plants show highly reduced expression of OsMADS50/SOC1 and for RFT1 (Rao et al., 2008), and we show here RFL can bind and induce OsSPL14 expression we suggest the RFL-OsSPL14 module can contribute to the transition of the SAM to flowering. Further, OsSPL14 in the young panicles directly activates *DENSE AND ERECT PANICLE1* (*DEP1*) to control panicle length (Lu et al., 2013). Thus RFL-OsSPL14-DEP1 module could explain the role of RFL in controlling panicle architecture (Rao et al., 2008; Ikeda-Kawakatsu et al., 2012). Thus RFL plays a role in floral transition and this function is conserved across several LFY homologs.

Our data ChIP-seq in the wild type tissue and gene expression modulation studies in transgenics also give molecular evidences for the role of RFL in suppression of floral fate. The direct binding of RFL to *OsMADS17*, *OsYABBY3*, *OsMADS58* and *HD-ZIP-IV* loci and the changes in their transcript levels on induction of RFL support this hypothesis. Once the transition from SAM to FM takes place, we

speculate RFL represses the conversion of inflorescence branch meristems to floral fate by negatively regulating *OsYABBY3*, *HD-ZIP class IV* and *OsMADS17* that can promote differentiation. These hypotheses indicate a diverged function for RFL in floral fate repression. *Arabidopsis* LFY is known to activate the expression of *AGAMOUS* (*AG*), whose orthologs in rice are *OsMADS3* and *OsMADS58*. Our studies confirm conservation with regard to RFL binding to *cis* elements at *OsMADS58* locus that is homologous to Arabidopsis *AG*. But importantly we show altered consequences of this binding on gene expression. We find RFL can suppress the expression of *OsMADS58* which we speculate can promote a meristematic fate. Further, we also present the abnormal upregulation of floral organ fate genes on RFL downregulation. These data too indicate functions of RFL, are in part, distinct from the role of *Arabidopsis* LFY where it works in promoting floral meristem specification and development. These inferences are supported by our data that rice gene homologs for *AP1*, *AP3* and *SEP3* are not directly regulated by RFL, unlike their direct regulation by *Arabidopsis* LFY during flower development.

We also report the expression levels of *LAX1*, *FZP*, *OsIDS1* and *OsMADS34* genes involved in meristem phase change and IM branching are RFL dependent. This is consistent with its role in the suppression of determinacy, thereby extending the IM activity for branch formation. But as yet we do not know if these effects are direct. Together, our data report direct targets of RFL that contribute to its functions in meristem regulation, flowering transition, and suppression of floral organ development. Overall, our preliminary data on RFL chromatin occupancy combined with our detailed studies on the modulation of gene expression provides evidence for targets and pathways unique to the rice RFL during inflorescence development.

**Comparative analysis of genes downstream to RFL in vegetative tillers Vs panicles**

Tillers and panicle branches arise from the axillary meristems at vegetative and reproductive stages, respectively, of a rice plant and overall contribute to the plant

architecture. Some regulatory factors control branching in both these tissues - for example, MOC1 and LAX1. Mutants at these loci affect tillers and panicle branch development thus indicating common mechanisms control lateral branch primordia development (Li et al., 2003; Komatsu et al., 2003; Oikawa and Kyozuka, 2009). Knockdown of RFL activity or loss-of-function mutants cause significantly reduced panicle branching and in few instances, reduction in vegetative axillary branching (Rao et al., 2008; Ikeda- Kawakatsu et al., 2012). We took up the global expression profiling of RFL knockdown plants compared to wild type plants in the axillary meristem and branching panicle tissue. These data provide a useful list of potential targets of RFL in axillary meristem and branching panicle tissue. The comparative analysis of the genes affected in the two tissues indicates only a subset of genes is affected by RFL in both the vegetative axillary meristems and branching panicle. These genes include transcription factors (*OsSPL14*, Zn finger domain protein, and bHLH domain protein), hormone signalling molecules (GA2 ox9) and cell signalling (LRR protein) as a set of genes activated by RFL in both tissues. On the other hand, these comparative expression profiling studies also show distinct set of genes deregulated by *RFL* knockdown in these two tissues therefore implicating RFL functions have a tissue-specific context. The genes deregulated only in axillary meristem tissue only include *D3*- involved in the perception of strigolactone, *OsMADS34* speculated to have a role in floral transition and *RCN1* involved in transition to flowering. On the other hand, the genes – *CUC1*, *OsMADS3*, *OsMADS58* involved in organ development and floral meristem determination were found to be deregulated only in panicle tissues of *RFL* knockdown plants. These data point towards presence of distinct mechanisms for the development of AMs as tillers versus the development of panicle axillary as rachis branches. Overall, these data implicate genes involved in transition to flowering, axillary meristem development and floral meristem development are controlled by RFL in different meristems to thereby control plant architecture and transition to flowering.